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THE MICROBIOLOGY OF BUTTER

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A Thesis
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INTRODUCTION.

In 1931 the total value of all dairy produce in Canada was estimated at $161,243,580*. Of this amount, $50,198,878 was the value of creamery butter. Dairy production in Alberta in the same year was valued at $16,572,612. The value of creamery butter produced there during this period amounted to $4,711,000. In the previous year the butter and cheese industry was placed sixth among the leading Canadian industries based on the total net income.

This large creamery industry has up to the present time made very little use of bacteriological tests for quality. One test, the plate counts of yeasts and molds, has been applied to some extent. As far as can be determined, little critical research on the value of this test has been conducted. The estimation of yeasts and molds as a guide to quality is not used as a microbiological test in other dairy products. In these cases the plate count of bacteria, or some test based on the bacterial content, is used. It is desirable, therefore, that some investigation be made of the value of the yeast and mold count, of the variability of the plate count of yeasts and molds and of the factors influencing the growth of yeasts and molds in butter.

*Canada Year Book, 1933.

THE LITERATURE ON THE MICROBIOLOGY OF BUTTER.

A perusal of the literature on this topic failed to reveal that any investigation has been made of the variability of the plate count of microorganisms in butter. It seems possible that the accuracy of such counts has not been questioned to the same extent as has been the case with regard to milk. This is probably due to the comparatively small amount of bacteriological control work which has been done on butter. In addition, microbiological results have not been used as a basis for grading any except exhibition butter.

A study of the variability of yeasts and molds in butter and of the methods used in such analyses is desirable before any further standards are proposed for classifying butter on the basis of the yeast and mold count. The classifications which have been proposed for use in the yeast and mold control of butter do not appear to be based on experimental data. The Canadian modified score card for exhibition butter contains such a classification of the plate counts of yeasts and molds with 25 classes. In the first ten classes the range of each class is only 10. It will be shown in this paper that this margin is probably within the range of the variability of the petri plate method.

The variability of the plate counts of milk has been recognized for a number of years. Among the first reports stressing this variability is that of Breed and Stocking (2). They point out that a plate count of bacteria at its best is only an estimate and should not be used in making fine distinctions. Brew and Dotterrer (4) make the following statement on the plate counts of milk: "The "groups" of bacteria in milk break apart on the average into 2.6 smaller "groups" in the process of preparing dilution waters. That is, under usual conditions the plate count will be increased from 2 to 3 times as a result of this factor alone. In individual cases, however, it may be increased more than this, the highest increase noted of the plate count is affected both because of the variation in the size of the "groups" and also because the clumps do not entirely separate into their component individuals during the plating process". Breed and Stocking (3) report a co-operative experiment between two laboratories on the variability of the plate count of milk. Two types of milk were studied; market milk and milk with a low bacterial content inoculated with a large number of Escherichia coli which display a minimum clumping tendency. The plate counts of the inoculated milks had a coefficients of variability between 7.7 and 13.8 percent. Large variabilities for market milk,

especially samples containing streptococci were shown, however. These authors make the following statement: "- - - - - - - - - ,a margin of one to five is usually sufficient to eliminate any chance that the lower "count" represents the greater number of bacteria, but even this margin does not appear to be sufficient to cover the errors introduced through the clumping of streptococci".

The data presented by Breed and Stocking above, have been analysed by Fisher, Thornton and Mackenzie (8). They found that in series 'B' and 'C', in which inoculations of coli were used, the plate counts varied in the same manner as samples from a Poisson Series.

Supplee, Whiting and Downs (24) conceived the idea of preparing a population of 100 counts from one dilution of a sample of milk in a study of the variations in bacteria counts caused by uneven temperatures of incubation.

Wright and Thornton (27) prepared replicate plates from carefully made dilutions of milk. The plates were numbered in the order of preparation. The populations obtained from a number of milks in this manner were studied statistically to determine the variability of the plate count. The average coefficient of variability for all counts was 25 percent. The high and low extremes were 57 percent and 15 per-

cent. They claimed that a variation of less than 100 percent between two dilutions of the same milk could not be depended on. Mudge and Lawler (17) made a similar study of the plate counts of 9 milks. Their graphs show a large increase in the counts following the 40th plate. For the 9 samples of milk reported, the average coefficient of variability for the first 40 plates was 32 percent. For the entire 75 plates it was 62.9 percent. As a result of these observations the authors stress the importance of the apparent instability of bacterial clumps in a solution of milk in water. They suggest that errors from this factor may be reduced by plating dilutions as quickly as possible or within the 20 minute period specified in 'Standard Methods of Milk Analysis'. Mudge and Lawler in another paper (18) report further work in which different methods of investigation were used. In these experiments a separate dilution blank was used for each replicate plate. By this method increases which they detected following the 40th plate in their previous work were prevented. By this technique the coefficient of variability averaged 18.5 percent. A dilution blank containing a buffer solution at pH 6.6 had a similar effect.

Albus (1) while recognizing the inaccuracies in bact-

erial enumerations lays much emphasis on errors caused by faulty technique in making plate counts. This author apparently does not recognize as important the inaccuracies which may be introduced by a factor such as the clumping of bacteria.

GENERAL PROCEDURE.

Procedure for Parts One and Two.

Material:

Many of the samples used were sent to the laboratory for yeast and mold analysis. Unless otherwise stated, the samples were removed with sterile spatulas from two corners of 56 pound boxes, thus insuring the inclusion of a portion of the surface. The samples were stored in sterile 4 oz. screw top jars.

Preparation of Samples:

Butter samples were melted in a water bath held at 42°- 45° C. and were plated as soon as the butter reached a consistency which would permit the use of a pipette. Some preliminary tests proved that the melted butter samples did not show a significant increase or decrease in the plate counts of yeasts, molds or bacteria when maintained at 42° to 45° C. for three hours.

Plating:

The pipettes used were straight-walled, 1 c.c. pipettes which are used in milk analysis. Warm pipettes were used to facilitate the removal of butter. The butter samples were always well mixed before removing any of the butter. The

usual procedure was to shake the sample 25 times through a distance of one foot. Dilution blanks of physiological salt solution or tap water were used in all the experiments and losses due to sterilization were made up by the aseptic addition of sterile water. In most of the analyses the medium was poured within 20 minutes after making the dilutions. In every case the dilution blanks were shaken according to 'Standard Methods of Milk Analysis' (23).

When a number of replicate plates were prepared from one sample, the same general procedure was followed except that the dilution blank was reshaken and another pipette was substituted after each set of ten plates. When a series of replicate plates was made from a sample of butter without dilution, large samples, usually 4 ounces, were used in place of dilutions and were handled in the same manner as diluted samples.

Medium:

The medium used for the yeast and mold counts throughout the experiments was Bacto dehydrated malt agar, acidified to pH 3.5 ± 0.1. This acidity is recommended by Hood and White (10) in which communication they report that at pH 3.5 it is certain that any variability due to the growth of bacteria is removed. Clerkin (6) confirms the results reported by Hood and White. My own preliminary results

have shown that at pH 3.8, larger and more easily counted colonies are obtained. However, pH 3.5 ± 0.1 was employed due to the adoption of this acidity in standard procedure. At first the pH of the medium was checked colorimetrically, using Bromphenol Blue as an indicator. This method was soon discarded in favor of the electrometric method* due to the difficulty of reading an indicator in a colored medium. The potentiometric method with a saturated calomel cell and a quinhydron electrode was employed on the solidified agar at room temperatures in the determinations of pH for check purposes. The medium was sterilized in 100 and 300 c.c. quantities and acidified just prior to plating with a 1:10 dilution of U. S. P., 85 percent lactic acid. This dilution of acid gives approximately a 1 normal solution of lactic acid. The amount of acid specified by the manufacturers of the medium was, in some cases, insufficient to increase the acidity to pH 3.5. For the bacteria counts standard nutrient agar was employed. The acidity was adjusted between pH 6 - pH 7.

Pouring the Medium:

Prior to pouring the medium, the petri dishes were plac-

*The author is indebted to the National Research Council for the loan of apparatus with which the potentiometric determinations reported in this paper were made.

ed on a warm plate in order to melt the butter, enabling thorough mixing of the butter with the medium. The warm plate was heated by water at approximately 60°C. An air space was maintained between the hot water and the warm plate which prevented the inoculum in the petri dishes reaching a temperature in excess of 40°- 45°C. This temperature is insufficient to destroy yeasts and molds while the medium is being poured.

Incubation:

The yeast and mold plates were incubated at 25°C. for 5 days, which is the recommended procedure of Hood and White (11) and the preliminary butter report (20). This procedure was found satisfactory except when a large number of molds were present, in which cases it appeared preferable to count in three days. Plates containing a large number of molds, when incubated for a period of 5 days at 25°C., were difficult to count with accuracy as the mold colonies merge together and lose their identity. Bacteria plates were incubated at 25°C. for 4 days.

Counting:

The counting of yeast and mold colonies was done with the naked eye aided by light passing through the plate. A

lumi-lens plate counter proved unsatisfactory for counting yeast and mold colonies especially in plates onwhich dilutions were not made, due to the dispersion of fat droplets throughout the medium. Bacteria counts were made with the aid of a lumi-lens plate counter. All counting was done with the assistance of a hand tally. Plate counts are reported on a per cubic centimeter basis.

Procedure for Part Three.

Material:

One-pound prints of butter, low in mold content, were used in the experiments reported in Part Three. The one-pound prints were divided into two equal parts. One half was used without inoculation and the remaining half was inoculated with mold spores. Each half was then further subdivided into sections of approximately one-tenth of a pound each.

Inoculation:

Two cultures of Penicillium, which is a common cause of surface mold on butter, were isolated from moldy parchment paper and employed for the inoculations. For inoculation purposes old slants containing an abundance of spores were used. Following inoculation the butter was well mixed

to distribute the spores.

Preparation of Parchment:

The parchment paper used in these experiments was prepared by the following methods. Saturated solutions of salt (NaCl) were made up in which the wrappers were soaked. Some of the wrappers soaked in saturated salt solution were raised to and maintained at 97°C. for an hour. In the cases where heat was not employed, the wrappers were allowed to soak in the saturated salt solution for several hours. The autoclaved wrappers were dampened in water before sterilization. The parchment used in the dry condition was taken directly from the manufacturer's package.

Due to the fact that the action of specific molds was not being studied no precautions were taken to maintain aseptic conditions when wrapping the butter.

Storage of Samples:

In preliminary experiments the wrapped portions of butter were placed together in a humidifier. This method was discontinued due to the diffusion of moisture and salt from one package to another by the moisture of condensation. In the experiments reported each sample was placed in an individual sample jar and stored in a moist chamber. In addition 2 c.c. of water were added to each jar to insure a

saturated atmosphere. The moist chambers consisted of large bell jars placed over a surface of water. The bell jars were stored in an ice box at 12°- 15°C.

Samples of each butter, before and after inoculation, were taken for salt and moisture analyses and mold counts.

PART ONE.

THE VARIABILITY OF THE PLATE COUNT IN THE ENUMERATION OF MICROORGANISMS IN BUTTER.

INTRODUCTION.

In this section of the research an attempt has been made to study the variability displayed by the plate counts of microorganisms in butter. The research which has been reviewed on the plate counts of milk has revealed considerable variability in the estimation of bacteria by this method. The inaccuracies which have been demonstrated do not invalidate this method for the estimation of microorganisms. This information has instead resulted in a more intelligent interpretation of plate counts. It seemed probable that the plate counts of microorganisms in butter would display a variability similar to that reported for the bacteria in milk. Some information on this variability is necessary in order to prevent any misconception of the accuracy with which estimates of the numbers of microorganisms in butter can be made.

The Variability of Replicate Plate Counts of Microorganisms from the same Sample of Butter.

The technique in which a number of replicates are prepared from one sample has been employed in this study of variability. Fifty replicate plates each of samples of commercial butter were prepared. A preliminary analysis was made in each case in order to determine the correct dil-

utions required. The populations obtained by this method have been used to measure the variability of the plate count method when it is applied to butter.

Three common measures of dispersion have been calculated for each population of replicate plates, namely: the high and low counts, average deviation and standard deviation. The coefficient of variability has also been used in comparing the variabilities.

Comsiderable criticism has often been levelled at the use of the statistical method on data of the type to be reported. This criticism has probably been justified in many instances. However, some measure must be employed in measuring the variability of plate counts and this method is probably the most satisfactory. In the data to be presented the populations of replicates are through necessity quite small from a statistical point of view. The frequency distributions obtained show a considerable amount of skewness or lack of symmetry. There is, however, in a few cases an approach to a symmetrical distribution of the "normal" type which suggests that if more data were available such a distribution might be approximated. These facts make it obvious that if the statistical method is applied, care must be taken in the interpretation of the results. If this

warning was always heeded much of the criticism of the application of the statistical method to such data would be removed. Too many investigators of biological phenomena have not observed these facts and consequently have endeavored to express their observations in exact mathematical terms which in many cases have not been justified by the data which were analyzed.

Table 1 contains a summary of the variability in the replicate combined counts of yeasts and molds. A wide range between the high and low of each butter is seen. The high counts vary approximately two to five times the value of low counts. The extent of the dispersion as measured by the average and standard deviations indicates considerable spread on each side of the mean value. The coefficients of variability are approximately the same value as those reported for bacteria counts in milk by the use of similar methods. The data in this table are rearranged in Table 2, in which averages of five replicate plates are arranged in the order of plating for each butter. The results indicate that in the high count samples in which dilutions were used there is a gradual increase in the plate count as the plating proceeds. This increase is similar to that reported by Mudge and Lawler for the plate counts of bacteria in milk with the exception that the increase noted here is a gradual one from

TABLE 1.

STATISTICAL CONSTANTS FOR THE COMBINED PLATE COUNT OF YEASTS AND MOLDS OF 8 BUTTERS BASED ON 50 REPLICATE PLATES EACH.

Butter Number	Dilution	High Count	Low Count	Mean	Average Deviation from the Mean	Standard Deviation	Coefficient of Variability
1*	1:10	1,160	400	822 ± 7	125.5	112 ± 5	13.6%
2	1:10	650	220	470 ± 9	92.2	95 ± 6	20.2"
3	1:10	1,070	450	698 ± 13.7	118.2	143.6 ± 9.7	20.6"
4	1:10	470	190	326 ± 7	50.6	69.2 ± 5	21.2"
5	No dilution	88	41	61 ± 2.4	10.6	25.2 ± 1.7	47.6"
6	" "	27	8	16.9 ± 0.4	3.1	3.9 ± 0.3	23.1"
7	" "	20	4	10.9 ± 0.4	3.1	3.9 ± 0.3	35.6"
8	1:10	270	80	169.6 ± 5.6	43.6	54 ± 3.6	31.9"
Averages		469	174				26.7

* 100 replicates.

TABLE 2.

COMBINED PLATE COUNTS OF YEASTS AND MOLDS
OF 8 BUTTERS BY AVERAGES OF FIVE PLATES ARRANGED IN THE ORDER OF PLATING.

Butter Number	AVERAGE PLATE COUNT									
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
1	500	500	740	790	730	830	870	910	780	800
2	320	260	480	450	540	500	490	520	540	580
3	500	490	650	660	740	680	850	790	870	750
4	280	220	350	300	330	370	370	320	340	370
5	63	55	74	64	70	74	68	62	50	69
6	15	20	19	16	18	19	16	13	17	17
7	13	11	10	14	11	9	10	8	10	9
8	100	120	180	160	160	200	170	220	210	180
Average 1,2,3,4,8.	340	318	480	472	500	516	550	552	548	536
Average 5,6,7.	30	28	34	31	33	34	31	28	26	32

the start of plating. These investigators secured evidence that the increases they observed were caused by a dispersion of clumps of bacteria in the dilution water. Due to the similarity in the method of reproduction of yeasts and bacteria the increases observed in Table 2 may also be due to a dispersion of clumps of cells.

Results similar to those in Tables 1 and 2 are reported for the yeast count in Tables 3 and 4. In each case the high count is less than five times the low count with the exception of sample 4 in which the high count is seven times the low count. The average coefficient of variability, 25.7 percent, is of the same order of magnitude as that obtained for the combined counts in Table 1. The increase in the plate count of high count butter in which dilutions were employed, observed in Table 2, did not occur in a few of the samples reported in Table 4.

The mold counts in Table 5 show a greater percentage range between high and low extremes in some samples than that observed for yeast counts. The dispersion of counts reported for molds is greater than that observed on the combined count in Table 1 or the yeast count in Table 3. The greater dispersion of counts has resulted in a coefficient of variability which is nearly twice as large as that reported in Tables 1 and 3. In Table 6 no increases in counts

TABLE 3.

STATISTICAL CONSTANTS FOR THE PLATE COUNT OF YEASTS OF 14 BUTTERS BASED ON 50 REPLICATE PLATES EACH.

Butter Number	Dilution	High Count	Low Count	Mean	Average Deviation from the Mean	Standard Deviation	Coefficient of variability
1	1:10	500	130	325.3 ± 8.8	16.3	91.3 ± 6.2	28.1%
2	1:1000	54,000	23,000	39,520 ± 602	5,060	6,310 ± 426	15.9"
3	1:10	490	160	331.6 ± 7.3	59.6	76.8 ± 5.2	23.2"
4	1:10	210	30	109 ± 3.6	35.7	37.9 ± 2.6	34.8"
5	1:100	4,200	1,900	2,730 ± 58	400	611 ± 41	22.4"
6	1:10	440	90	294 ± 7.6	65.8	80 ± 5.4	27.2"
7	1:10	420	170	299.6 ± 6.7	61.8	70.4 ± 4.7	23.2"
8	1:10	360	140	266.8 ± 5.5	54.7	58.0 ± 3.9	21.2"
9	No dilution	28	7	14.4 ± 0.4	3.5	4.3 ± 0.3	29.8"
10	" "	84	40	65.1 ± 1.2	11.4	12.4 ± 0.8	19.0"
11	" "	23	6	15.2 ± 0.3	3.6	3.6 ± 0.2	23.8"
12	" "	14	3	7 ± 0.3	2.7	2.9 ± 0.2	42.1"
13	" "	25	6	12.5 ± 0.4	2.7	3.9 ± 0.3	31.5"
14*	1:10	1,130	380	828.3 ± 10.3	115.9	153.2 ± 7.3	18.1"
Averages		4,423	1,862				25.7

* 100 replicates.

TABLE 4.

PLATE COUNTS OF YEASTS OF 14 BUTTERS BY AVERAGES OF FIVE PLATES ARRANGED IN THE ORDER OF PLATING.

Butter Number	AVERAGE PLATE COUNT									
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
1	190	230	310	360	360	380	330	290	400	320
2	42,000	37,000	39,000	42,000	41,000	39,000	42,000	38,000	35,000	39,000
3	240	240	350	350	350	280	360	360	360	420
4	100	70	110	100	100	130	120	120	110	120
5	3,000	2,700	2,500	2,500	2,900	2,800	2,900	2,600	3,000	2,400
6	170	140	270	270	320	260	310	290	300	360
7	220	230	310	280	300	300	330	310	360	270
8	230	160	260	230	240	280	290	230	240	280
9	12	10	16	14	14	15	17	15	16	14
10	59	52	70	60	65	69	68	61	50	63
11	13	18	16	14	16	18	14	12	16	15
12	9	7	10	9	7	7	6	5	5	5
13	13	11	16	14	15	10	12	10	10	13
14	480	480	710	760	700	780	820	870	720	770
Average	3,338	2,953	3,139	3,354	3,313	3,161	3,391	3,084	2,899	3,146

TABLE 5.

STATISTICAL CONSTANTS FOR THE PLATE COUNT OF MOLDS OF 10 BUTTERS BASED ON 50 REPLICATE PLATES EACH.

Butter Number	Dilution	High Count	Low Count	Mean	Average Deviation from the Mean	Standard Deviation	Coefficient of variability
1	1:1000	43,000	19,000	29,980 ± 641	5,560	6,720 ± 453	22.4%
2	1:1000	80,000	48,000	63,540 ± 640	5,620	6,750 ± 450	10.8"
3*	1:10	90	10	34.4 ± 1.2	31.2	18.2 ± 0.9	52.9"
4	1:1000	25,000	9,000	15,960 ± 370	3,400	3,960 ± 270	24.8"
5	1:10	80	10	42.4 ± 1.5	11.1	16.3 ± 1.1	38.5"
6	No dilution	9	0	3.5 ± 0.2	1.6	2.1 ± 0.1	58.5"
7	" "	4	0	1.3 ± 0.1	1.8	1.7 ± 0.1	130.7"
8**	1:10000	130,000	10,000	53,400 ± 2,890	23,800	30,333 ± 2,040	56.7"
9**	1:10000	220,000	10,000	72,600 ± 3,520	27,700	36,690 ± 2,488	50.8"
10**	1:20000	2640000	1520000	1995200 ± 24600	210600	259200 ± 17400	12.9"
Averages		313,818	161,602				45.8

* 100 replicates.
** Artificially inoculated.

TABLE 6.

PLATE COUNTS OF MOLDS OF 10 BUTTERS BY AVERAGES OF FIVE PLATES ARRANGED IN THE ORDER OF PLATING.

Butter Number	AVERAGE PLATE COUNT									
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
1	35,000	33,000	34,000	29,000	34,000	25,000	30,000	27,000	27,000	26,000
2	63,000	59,000	66,000	61,000	64,000	63,000	69,000	64,000	66,000	60,000
3	20	20	30	30	30	50	40	40	20	30
4	18,000	19,000	19,000	19,000	13,000	19,000	13,000	14,000	19,000	13,000
5	40	40	40	30	50	50	40	40	40	40
6	4	3	4	6	5	4	3	3	2	2
7	1	1	3	1	1	1	1	1	0	2
8	60,000	30,000	40,000	100,000	70,000	20,000	40,000	50,000	70,000	50,000
9	70,000	90,000	120,000	70,000	60,000	70,000	70,000	60,000	70,000	70,000
10	2300000	2280000	1942000	2000000	1980000	1920000	1980000	1840000	1780000	1640000
Ave.	254,606	251,106	222,107	227,906	222,108	211,710	220,208	205,508	203,206	185,907

were obtained as the plating proceeded. This result suggests that the increases noted in Table 2 are due to the yeasts. In some samples of butter a decrease in the plate count was obtained as the plating of replicates proceeded.

In addition to the yeast and mold counts, bacteria counts were made in replicate on 9 butters. The results are summarized in Table 7. Some of the samples show little variability. The percentage range between high and low counts is less than that observed for yeasts and molds. The average coefficient of variability is about the same as that obtained on the yeast count and the combined count of yeasts and molds. No increases for the five plate averages of replicate plates are observed in Table 8. In some samples a decrease rather than an increase is observed.

The results in the preceding Tables show a high variability in the plate counts of microorganisms in butter when replicate plates are prepared from each sample. The coefficient of variability suggests that the mold counts of butter on replicate plates are probably two times as variable as similar counts of combined yeast and mold counts and bacteria counts. The dispersion of counts obtained confirm the observation of Breed and Stocking that a margin of one to five is usually sufficient to eliminate any chance that the lower "count" represents the greater number of bacteria.

TABLE 7.

STATISTICAL CONSTANTS FOR THE PLATE COUNT OF BACTERIA OF 9 BUTTERS BASED ON 50 REPLICATE PLATES EACH.

Butter Number	Dilution	High Count	Low Count	Mean	Average Deviation from the Mean	Standard Deviation	Coefficient of Variability
1*	1:1000	170,000	113,000	142,800 ± 670	8,860	6,930 ± 470	4.8%
2	"	116,000	72,000	92,480 ± 925	7,500	9,700 ± 654	10.5"
3*	"	104,000	66,000	83,160 ± 5,030	7,490	52,800 ± 3,600	63.5"
4	"	46,000	24,000	34,260 ± 451	4,210	4,700 ± 319	13.8"
5	"	87,000	46,000	63,940 ± 920	7,920	9,710 ± 650	15.3"
6	"	78,000	40,000	59,700 ± 740	6,520	7,780 ± 520	13.0"
7	"	46,000	18,000	28,160 ± 500	3,890	5,240 ± 353	18.9"
8	"	47,000	21,000	33,700 ± 1,460	4,940	15,340 ± 1,035	45.5"
9	"	36,000	13,000	22,000 ± 540	4,740	5,750 ± 387	26.1"
Averages		81,111	45,888				23.5

* 49 replicates.

TABLE 8.

PLATE COUNTS OF BACTERIA OF 9 BUTTERS BY AVERAGES OF FIVE PLATES ARRANGED IN THE ORDER OF PLATING.

Butter Number	AVERAGE PLATE COUNT									
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
1	152,000	145,000	145,000	144,000	149,000	129,000	139,000	140,000	143,000	136,000
2	92,000	92,000	97,000	93,000	92,000	99,000	89,000	89,000	87,000	93,000
3	92,000	92,000	77,000	83,000	85,000	92,000	80,000	80,000	73,000	78,000
4	40,000	35,000	36,000	35,000	31,000	33,000	32,000	35,000	34,000	33,000
5	77,000	70,000	66,000	63,000	62,000	66,000	59,000	58,000	60,000	58,000
6	61,000	56,000	50,000	62,000	61,000	60,000	63,000	57,000	64,000	50,000
7	30,000	29,000	26,000	30,000	26,000	27,000	32,000	26,000	28,000	26,000
8	35,000	38,000	37,000	35,000	34,000	31,000	31,000	29,000	32,000	33,000
9	27,000	26,000	24,000	27,000	23,000	22,000	18,000	16,000	15,000	17,000
Average	67,333	64,777	62,000	63,555	62,555	62,111	60,333	58,888	59,555	52,222

There have been some fluctuations in the counts of replicates in which the high count was seven times as large as the low count. These cases have been extremes. The average and standard deviations are more accurate measures of the whole dispersion. Mudge and Lawler have shown that by a dispersion of clumps of bacteria in the dilution blanks the coefficients of variability have been greatly increased. The results of this research show that a considerable amount of variability exists when there is little indication that clumps of cells have been dispersed during the plating process.

Two distributions from the data reported in Tables 1 to 8 were analyzed statistically for goodness of fit with a normal distribution. The data on sample 2, Table 7, for plate counts of bacteria fit a normal distribution very well. The other distribution, sample 1, Table 1 is poorly fitted by a normal curve. This result is interesting in view of the fact that Fisher, Thornton and Mackenzie report that the data of Breed and Stocking on replicate counts of milk constitute a Poisson Series. Although no attempt was made to fit the Poisson Series, the fact that one distribution obtained on replicate plates of bacteria in butter was fitted well by a normal distribution indicates that various distributions are probably approached when a small number of re-

plicates are analyzed. It may be pointed out that the data analyzed by Fisher, Thornton and Mackenzie are not representative of normal milk. These data were obtained by Breed and Stocking on carefully selected low count milk which was inoculated with Escherichia coli in order to reduce errors caused by clumping. A different result would probably be obtained if an ordinary sample of milk was analyzed statistically in the same way.

Dilutions are often prepared in the plate counting of yeasts and molds in butters which are quite low in microorganisms. The reasons for this procedure are as follows:

(1) The acidity of the medium often causes the precipitation of the butter curd with the formation of particles which are often difficult to distinguish from subsurface colonies of yeasts. This is particularly true of plates containing over 75 colonies per plate. In addition to the difficulty of counting, a uniform distribution of the butter under these conditions is difficult to obtain due to the adherence of the precipitate to the petri dish.

(2) Due to the presence of globules of melted butter in the butter-agar emulsion it is almost impossible to count with accuracy the number of colonies usually counted per plate in milk analysis.

TABLE 9.

A COMPARISON OF THE NO-DILUTION AND DILUTION PLATE COUNTS OF YEASTS AND MOLDS OF 79 SAMPLES OF BUTTER.

Sample Number	PLATE COUNTS				Difference between Combined Counts
	No-Dilution		1:10 Dilution		
	Molds	Yeasts	Molds	Yeasts	
1	0	159	0	140	- 19
2	0	2	0	10	8
3	0	1	0	10	9
4	1	70	0	170	99
5	1	8	0	0	- 9
6	0	37	10	30	3
7	0	96	20	90	14
8	13	34	30	60	43
9	1	3	10	0	6
10	0	35	0	110	75
11	4	167	20	420	269
12	1	9	10	10	10
13	2	44	0	50	4
14	0	34	10	50	26
15	3	1	20	0	16
16	1	2	0	10	7
17	1	0	0	0	- 1
18	13	0	10	0	- 3
19	0	2	0	0	- 2
20	5	44	0	60	11
21	0	0	10	0	10
22	2	43	20	100	75
23	0	1	0	0	- 1
24	2	0	0	0	- 2
25	1	2	0	10	7
26	1	9	0	10	0
27	4	87	10	50	- 31
28	3	0	10	0	7
29	1	21	0	80	58
30	1	73	0	70	- 4
31	3	79	0	230	148
32	1	187	0	500	312
33	4	35	70	100	131
34	0	158	0	260	102
35	1	51	0	190	138
36	0	94	0	140	46
37	2	98	0	170	70
38	0	63	0	150	87
39	0	14	0	30	16
40	6	14	10	20	10

Continued.

TABLE 9.

Continued.

Sample Number	PLATE COUNTS No-Dilution Molds	No-Dilution Yeasts	1:10 Dilution Molds	1:10 Dilution Yeasts	Difference between Combined Counts
41	0	82	0	140	58
42	1	20	0	20	- 1
43	0	26	0	40	14
44	0	32	0	50	18
45	1	74	0	200	125
46	4	90	0	120	26
47	2	47	0	70	21
48	0	20	0	80	60
49	1	2	0	10	7
50	0	7	0	10	3
51	1	56	0	40	- 17
52	1	150	0	290	139
53	0	16	0	50	34
54	0	19	0	130	111
55	0	95	0	220	125
56	2	50	20	30	- 2
57	0	31	0	10	- 21
58	0	39	0	50	11
59	0	51	0	70	19
60	2	47	0	40	- 9
61	0	22	0	40	18
62	1	193	10	270	86
63	2	36	10	80	52
64	6	9	0	30	15
65	1	14	0	20	5
66	1	11	0	20	8
67	3	32	0	40	6
68	0	59	0	70	11
69	2	95	0	280	183
70	0	59	0	170	111
71	6	38	0	30	- 14
72	2	60	10	30	- 22
73	12	186	10	90	98
74	4	44	10	40	2
75	1	87	110	330	352
76	11	75	0	70	- 16
77	4	53	10	70	13
78	1	50	0	80	29
79	2	24	0	110	84

TABLE 10.

A COMPARISON OF THE COMBINED COUNT OF YEASTS AND MOLDS OF 79 SAMPLES OF BUTTER ON EACH OF WHICH COUNTS WERE MADE WITH AND WITHOUT DILUTION.

Range of No-dilution Counts	No. of Samples in each Range	COMBINED PLATE COUNT	
		No-dilution	1:10 dilution
1 - 10	17	3.8	8.2
11 - 20	9	16.0	44.4
21 - 30	5	23.4	58.0
31 - 40	9	35.5	68.8
41 - 50	8	47.1	63.7
51 - 60	8	54.7	93.7
61 - 70	2	65.5	95.0
71 - 80	3	73.3	146.6
81 - 90	4	84.5	440.0
91 - 100	7	95.3	157.1
150 - 200	7	174.1	287.1
Average		59.0	92.7

In the course of this research it has been observed that plate counts of diluted butter are higher than counts of the same butter undiluted. This observation points to the necessity for the adoption of uniform procedure in making dilutions in the analysis of butter for yeasts and molds. Table 9 contains the yeast and mold counts of 79 samples of butter on which counts were made with and without dilution. The counts of these samples indicate that by the process of dilution the yeast and mold counts on many samples are greatly increased. Only 17 samples in this Table show a slightly decreased count when dilutions were made; of this number, 7 are low count butters. The data have been summarized in Table 10. The averages obtained show that by the process of dilution the average combined count of yeasts and molds is approximately doubled. The reason for this result has not been determined. The same factor is probably responsible for the increases which are observed in Table 2, in which a steady increase of the yeast count was noted in certain samples of butter as a number of replicates were prepared.

Due to the extent to which the yeast and mold count has been lowered during the past few years most of the butter in Western Canada is reported to have low counts, a great many

of which approach zero. As a result of this trend toward the elimination of yeasts and molds in butter it may be expected that in a few more years many more zero counts will be reported. In view of these facts and the observation that the use of dilutions results in an approximate doubling of the combined count of yeasts and molds, one seems justified in proposing that a uniform procedure of no dilutions be employed in the routine yeast and mold analysis of butter. Any butters containing excessive numbers of yeasts and molds, which could not be estimated by this procedure, could be simply termed unsatisfactory from the standpoint of the yeast and mold count.

The Variability of the Plate Counts of Microorganisms from Different Boxes of the Same Churning.

In addition to the investigation into the variability displayed by the plate counts of microorganisms in one sample of butter an attempt was made to obtain some data on the distribution of microorganisms in a churning of butter. The fact that butter is solid makes it difficult to obtain a representative sample. The usual procedure, therefore, is to take a composite sample consisting of two portions from the package as described under 'Procedure'. The representativeness even of a composite sample of butter depends to a great extent on the distribution of microorganisms in the butter from which

the sample is taken. An uneven distribution of microorganisms due to some factors such as contamination from the equipment, especially the churn, or some condition favoring growth in certain portions may result in an inaccurate estimate of the microbiological condition.

Table 1 contains the data on 25 churnings of fresh and storage butter. Several boxes of each were examined. Composite samples were obtained by the procedure already mentioned. No finer distinctions than those reported below seem justified due to the variability of the petri plate method which has just been discussed.

The results show fluctuations of considerable magnitude in the plate counts of bacteria, yeasts and molds of a number of boxes from the same churning. The yeast and mold counts usually on churnings which show some zero counts, display minor fluctuations. Higher fluctuations were observed in the plate counts of butter with a slightly higher number of microorganisms and no zero counts. The exact significance of these results is difficult to explain due to the element of chance in sampling. In general, the results reported in Table 1 show fluctuations similar to those reported in the first part of this section and suggest that any differences observed must be discussed with caution as it is very likely that the var-

TABLE 1. THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA ON A NUMBER OF BOXES FROM EACH OF 25 CHURNINGS.

Churning No.	Organism	BOX NUMBERS 1	2	3	4	5	6	7	Average	High Count	Low Count	Range Between High and Low Count	The Range as Percent of Low Count
1	Bacteria	600	1,200	2,000	1,000	750	900	900	1,050	2,000	600	1,400	234
	Yeasts	12	14	45	16	16	12	25	20	45	12	33	275
	Molds	1	1	0	1	1	2	0					
2	Yeasts	12	4	24	8	8	4		10	24	4	20	500
	Molds	1	1	1	0	3	1						
3	Bacteria	11,350	9,050	8,850	27,250	7,950	8,700		12,192	27,250	7,950	19,300	243
	Yeasts	13	2	2	8	6	1		5.3	13	1	12	1200
	Molds	0	0	0	0	0	0						
4	Bacteria	61,000	49,000	41,000	17,000	60,000	13,700		40,284	61,000	13,700	47,300	345
	Yeasts	150	120	100	90	40	100		100	150	40	110	275
	Molds	100	50	80	110	10	110		77	110	10	100	1,000
5	Bacteria	80,000	22,000	81,000	26,000	45,000			50,800	81,000	22,000	59,000	269
	Yeasts	4	2	5	0	3			2.8	5	0	5	500
	Molds	4	4	3	1	0			2.4	4	0	4	400

CONTINUED.

TABLE 1. THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA ON A NUMBER OF BOXES FROM EACH OF 25 CHURNINGS.

CONTINUED.

Churning No.	Organism	BOX NUMBERS 1	2	3	4	5	6	7	Average	High Count	Low Count	Range Between High and Low Count	The Range as Percent of Low Count
6	Bacteria	18,200	14,600	14,800					15,900	18,200	14,600	3,600	25
	Yeasts	41	30	24					32	41	24	17	71
	Mycoderms	30	19	9					19	30	9	21	233
7	Bacteria	950	2,150	900	650	1,350	1,700		1,283	2,100	650	1,450	224
8	Bacteria	2,000	6,100	2,300	3,500	50,000	3,200	77,000	20,582	77,000	2,000	76,000	3,800
	Yeasts	114	53	169	24	61	181	87	98.5	181	24	157	654
	Molds	8	5	4	2	4	4	11	5.4	11	2	9	450
9	Bacteria	4,200	10,000	2,400	4,800	4,000			5,100	10,000	2,400	7,600	316
	Yeasts	0	0	0	0	0			0	0	0	0	
	Molds	1	1	0	1	1			1	1	0	1	
10	Bacteria	4,500	5,050	5,950	6,050				5,387	5,950	4,500	1,450	32
	Yeasts	1	0	0	0								
	Molds	0	0	0	0								

CONTINUED.

TABLE 1. THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA ON A NUMBER OF BOXES FROM EACH OF 25 CHURNINGS.

CONTINUED.

Churning No.	Organism	BOX NUMBERS 1	2	3	4	5	6	7	Average	High Count	Low Count	Range Between High and Low Count	The Range as Percent of Low Count
11	Bacteria	9,600	26,850	14,250	16,550				16,812	26,850	9,600	17,250	180
	Yeasts	1	4	20	3				7	20	1	19	1,900
	Molds	1	0	0	0								
12	Bacteria	10,000	8,000	10,400	7,550				8,987	10,400	7,550	2,850	265
	Yeasts	115	101	136	103				114	136	101	35	34
	Molds	1	2	3	1				2	3	1	2	
13	Bacteria	20,800	23,000	Spreader	20,300				21,967	23,000	20,300	2,700	13
	Yeasts	0	1	0	0								
	Molds	0	0	0	1								
14	Bacteria	6,050	5,400	6,700	6,600				6,187	6,700	5,400	1,300	24
	Yeasts	0	1	2	1								
	Molds	1	0	1	1								
15	Bacteria	4,600	4,650	5,550	3,750				4,633	5,550	3,750	1,800	48
	Yeasts	0	1	0	0								
	Molds	0	0	0	0								

CONTINUED.

TABLE 1. THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA ON A NUMBER OF BOXES FROM EACH OF 25 CHURNINGS.

CONTINUED.

Churning No.	Organism	BOX NUMBERS 1	2	3	4	5	6	7	Average	High Count	Low Count	Range Between High and Low Count	The Range as Percent of Low Count
16	Bacteria	4,400	4,550	4,950	4,800				4,675	4,950	4,400	550	13
	Yeasts	0	1	1	0								
	Molds	1	2	0	0								
17	Bacteria	8,600	8,350	9,900	9,500				9,088	9,900	8,350	1,550	19
	Yeasts	2	1	1	4				2	4	1	3	300
	Molds	0	2	1	0								
18	Bacteria	44,550	33,800	20,500	35,950				33,700	44,550	20,500	24,050	118
	Yeasts	0	1	1	1								
	Molds	1	3	2	2				2	3	1	2	
19	Yeasts	0	0	1	1								
	Molds	3	2	3	3								
20	Yeasts	3	4	2	2				3	4	2	2	
	Molds	0	0	0	1								

CONTINUED.

TABLE 1. THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA ON A NUMBER OF BOXES FROM EACH OF 25 CHURNINGS.

CONTINUED.

Churning No.	Organism	BOX NUMBERS 1	2	3	4	5	6	7	Average	High Count	Low Count	Range Between High and Low Count	The Range as Percent of Low Count
21	Yeasts	265	290	170	270				249	290	170	120	71
	Molds	10	10	15	10								
22	Yeasts	1	0	2	1								
	Molds	0	0	0	0								
23	Yeasts	0	1	0	1								
	Molds	1	0	1	0								
24	Yeasts	1	1	0	0								
	Molds	0	1	1	1								
25	Yeasts	0	1	1	1								
	Molds	1	1	0	0								

TABLE 2.

THE YEAST, MOLD AND BACTERIA PLATE COUNTS
OF A NUMBER OF SAMPLES FROM TWO CHURNINGS.

Sample No.	BUTTER 1. Bacteria	Molds	Yeasts	BUTTER 2. Bacteria	Molds	Yeasts
1	136,000	115	645	33,500	50	360
2	136,500	115	550	15,000	45	485
3	141,000	80	655	17,000	40	350
4	132,000	155	640			
5	123,000	95	985			
6	124,500	120	800			
7	116,500	130	1,100			
8	140,000	95	625			
9	136,500	112	695			
10	147,000	130	1,400			
Average	133,300	115	810	21,833	45	398
High	147,000	155	1,400	33,500	50	485
Low	116,500	80	550	15,000	40	350
Range	30,500	75	850	18,500	10	135
% Range is of Low Count	27%	94%	155%	124%	25%	39%

TABLE 3.

THE PLATE COUNTS OF MOLDS OF A NUMBER OF REPLICATE SAMPLES FROM A BOX OF BUTTER STORED AT 12° to 15° C. FOR SEVERAL MONTHS

Sample Number	Plate Counts of Mold Duplicates		Average
1	1,400	1,600	1,500
2	4,100	2,700	3,400
3	900	1,100	1,000
4	6,800	7,400	7,100
5	3,800	4,200	4,000
6	7,100	6,000	6,500
Average	4,000	3,800	3,900

TABLE 4.

THE PLATE COUNTS OF BACTERIA OF A NUMBER OF REPLICATE SAMPLES FROM A BOX OF BUTTER STORED AT 12° to 15° C. FOR SEVERAL MONTHS

Sample Number	Plate Counts of Bacteria Duplicates		Average
1	47,000	51,000	49,000
2	23,000	10,000	16,000
3	139,000	214,000	176,000
4	52,000	35,000	43,000
Average	65,000	77,000	71,000

iability of the plate count accounts for a large percentage of the difference noted. It is indeed difficult to attempt a differentiation of the variability of the distribution of microorganisms in butter and the variability of the method employed in counting.

Plate counts of several samples from two churnings manufactured in the same churn are reported in Table 2. These samples were taken at the time the butter was being removed from the churn. The results are somewhat similar to those reported in Table 1.

Tables 3 and 4 contain the plate counts of molds and bacteria in a number of replicate samples from a 7 pound box of butter which was stored in an ice box at 12°- 15°C. for several months. There was a considerable increase in microorganisms during this period. A larger fluctuation in the counts of several samples was obtained than in the normal butter reported in Tables 1 and 2.

A number of boxes from the same churning which had been exposed to temperatures favoring growth is reported in Table 5. In this churning considerable variability in counts was obtained. It is probably safe to interpret these results as being partially caused by factors other than the variability of the petri plate method. The results suggest an uneven

growth of microorganisms in the different boxes of the churning.

TABLE 5.

PLATE COUNTS OF MICROORGANISMS ON A NUMBER OF BOXES FROM THE SAME CHURNING, ALL BOXES BEING EXPOSED TO CONDITIONS FAVORING GROWTH.

Box No.	Plate Count per c.c. Molds	Yeasts	Bacteria
1	1 gr	0	14,500
2	0	0	very high
3	very high	-	74,000
4	" "	-	276,000
5	18 gr	131	93,000

The results reported in this section indicate that a churning of butter, manufactured under sanitary creamery conditions and stored subsequent to manufacture at temperatures preventing the growth of microorganisms, will not display unusual fluctuations in the plate counts of various portions of the same butter. Any fluctuations which will occur appear to be of a similar magnitude to those obtained for the plate count method in a previous section. If the above conditions are not closely adhered to, plate counts falling short of estimating the actual microbiological condition may frequently be obtained.

SUMMARY.

1. A large variability has been observed in the replicate plate counts of microorganisms in a number of samples of butter.

2. The coefficients of variability obtained for the replicate plate counts of microorganisms in butter are of approximately the same order of magnitude as those reported for replicate plate counts of bacteria in milk.

3. The results secured for replicate plate counts of microorganisms show a large spread between the extremes. In some samples the high count was seven times as large as the low count.

4. The yeast counts of replicate plates show a gradual increase in some high count samples when placed in the order of preparation. These increases did not occur in replicate counts of molds and bacteria.

5. As a result of the variabilities herein reported, the classification at present employed for exhibition butter appears to have too many artificial divisions.

6. The data obtained by replicate plate counts of samples of butter form distributions which are asymmetrical. The departure from a perfectly symmetrical curve is not great

enough to invalidate the use of the common statistical measures of dispersion if fine comparisons of the results are avoided. One distribution of replicate plate counts of bacteria was closely fitted by a normal curve.

7. On the basis of the increases which are observed to take place when dilutions are employed in the analysis of butter for yeasts and molds it is urged that uniform procedure be adopted. It is suggested that the use of dilutions be discontinued due to the large number of low counts at present reported.

8. Greater variability was observed in the counts obtained from different boxes of the same churning when the butter was held at temperatures favorable for the growth of microorganisms.

PART TWO

A COMPARISON OF THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA IN BUTTER.

In Canada it has been assumed that the combined plate counts of yeasts and molds may be used as a measure of the sanitation surrounding the manufacture of butter. This assumption is based on the fact that yeasts and molds are destroyed by pasteurization at 170° degrees F. for ten minutes. Any subsequent contamination, it has been assumed, results in the addition of yeasts and molds, the numbers of which can be estimated by the plate count.

A few preliminary results in the present work indicated considerable fluctuation in the relationship between the numbers of yeasts and molds and bacteria in butter. In other words there appeared to be very little correlation between the plate counts of yeasts and molds and bacteria. If this condition is true the yeast and mold count falls short of measuring the microbiological quality of butter. In order to obtain further data on this relationship a number of samples have been analysed for yeasts, molds and bacteria. Table 1 contains the plate counts of bacteria and yeasts and molds of 46 samples of exhibition butter. A scrutiny of the table reveals little correlation between the bacteria, yeast and mold counts. The yeast counts do increase in some samples after the bacteria count reaches approximately 25,000 but there are so many samples showing low counts of yeasts in

TABLE 1.

A COMPARISON OF THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA IN EXHIBITION BUTTER.

Creamery No.	Bacteria	Molds	Yeasts
1	250	0	2
2	400	0	2
3	400	1	4
4	400	6	7
5	1400	0	1
6	1400	0	0
7	1600	0	0
8	1800	0	30
9	2000	0	12
10	2100	28	0
11	2100	28	0
12	3000	0	0
13	3500	0	0
14	3800	16	21
15	4100	8	0
16	4600	1	7
17	4600	1	157
18	4600	0	5
19	5600	1	8
20	5700	2	35
21	6100	0	0
22	6200	12	12
23	7200	0	16
24	7800	0	4
25	8700	17	0
26	8900	1	1
27	10600	11	8
28	12000	3	5
29	15800	1	47
30	18800	18	9
31	19700	1	93
32	21700	1	8
33	22100	1	8
34	22800	5	17
35	25000	0	140
36	29000	0	2140
37	31800	2	5
38	38000	1	19
39	44500	10	1680
40	55000	1	1900
41	68600	0	0
42	75400	4	26
43	130000	0	12000
44	136000	34	28
45	400000	0	480
46	over 1000000	0	0

TABLE 2.

A COMPARISON OF THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA IN COMMERCIAL BUTTER.

Creamery No.	Bacteria	Molds	Yeasts
47	650	1	12
48	900	0	25
49	2000	0	45
50	2400	0	0
51	3600	3	0
52	5600	0	0
53	6250	0	57
54	6500	1	3
55	9000	0	4
56	9600	1	0
57	10600	0	111
58	10900	2	2
59	12900	1	1
60	15000	45	485
61	15500	60	1120
62	15500	2	3
63	15800	0	7
64	17000	40	350
65	17700	0	3
66	18200	0	41
67	21400	10	940
68	25000	36	15
69	27250	0	8
70	33500	50	360
71	42000	4	47
72	44300	35	8
73	46500	2	230
74	48500	4	7
75	50000	55	250
76	55000	30	180
77	61000	0	150
78	79000	1	131
79	80000	4	4
80	93500	0	980
81	95500	0	270
82	106500	2	990
83	107500	15	4500
84	113000	80	310
85	145000	250	2350
86	196000	1	22
87	600000	1	210
88	1000000	0	0
89	1000000	0	12
90	over 1000000	4	31

this region that the correlation is lowered. On the average it may be said that a low bacteria count butter is characterized by a low yeast count, but the reverse is not necessarily true.

Similar data have been prepared on commercial butter to see whether similar results would be obtained. The data on fresh commercial butter are contained in Table 2. The bacteria counts are arranged in ascending order of magnitude to illustrate the correlation. The relationship is very similar to that obtained with exhibition butter. These data contain a sufficient number of low yeast and mold count samples with high counts of bacteria to make the value of the yeast and mold count questionable as a sole measure of the sanitary production of creamery butter.

Additional confirmatory evidence of the lack of relationship between the plate counts of bacteria and the counts of yeasts and molds is presented in Table 3 which contains comparative counts on 83 samples of butter from one creamery. The churn in this creamery is equipped with a removable butter worker which is extremely difficult to keep in a sanitary condition. The flora of butter manufactured in this type of churn is characterized by a high count of yeasts and mycoderms relative to the bacteria count. Mater-

TABLE 3.

THE RELATIONSHIP BETWEEN PLATE COUNTS OF MYCODERMS, YEAST AND BACTERIA ON 83 SAMPLES OF BUTTER FROM ONE CREAMERY IN WHICH THE CHURN WAS EQUIPPED WITH AN EXTERNAL BUTTER WORKER.

Sample No.	Bacteria	Mycoderms	Yeasts
1	1,000	0	30
2	1,000	0	300
3	1,000	0	230
4	2,000	0	16
5	2,000	0	10
6	3,000	1	23
7	3,000	2	23
8	3,000	5	17
9	3,000	9	14
10	4,000	1	63
11	4,000	20	60
12	4,000	0	620
13	4,000	40	320
14	4,000	11	34
15	5,000	0	1
16	5,000	12	80
17	5,000	10	20
18	6,000	36	21
19	6,000	7	9
20	7,000	30	5
21	9,000	3	20
22	9,000	29	27
23	9,000	0	40
24	9,000	0	230
25	10,000	52	44
26	10,000	50	710
27	11,000	30	460
28	11,000	20	440
29	11,000	21	129
30	11,000	0	188
31	11,000	0	380
32	13,000	20	610
33	14,000	40	290
34	15,000	3	430
35	15,000	0	38
36	16,000	28	25
37	16,000	20	20
38	16,000	0	130
39	17,000	36	70

Continued.

TABLE 3. Continued.

Sample No.	Bacteria	Mycoderms	Yeasts
40	17,000	0	8
41	18,000	0	47
42	18,000	0	26
43	18,000	0	290
44	19,000	1	25
45	19,000	4	39
46	19,000	10	230
47	20,000	0	140
48	21,000	1	52
49	23,000	0	190
50	24,000	0	650
51	24,000	40	450
52	24,000	30	130
53	26,000	1	69
54	27,000	0	110
55	28,000	19	26
56	29,000	10	6
57	30,000	9	29
58	30,000	0	22
59	30,000	11	95
60	33,000	10	10
61	37,000	10	91
62	38,000	0	160
63	39,000	0	150
64	43,000	1	11
65	44,000	1	13
66	46,000	1	13
67	50,000	0	10
68	54,000	0	81
69	56,000	0	140
70	56,000	0	100
71	61,000	50	120
72	65,000	26	1
73	95,000	4	180
74	100,000	20	20
75	132,000	10	350
76	136,000	0	110
77	160,000	2	0
78	160,000	0	2
79	170,000	4	32
80	188,000	0	23
81	286,000	30	10
82	325,000	10	30
83	428,000	10	550

TABLE 4.

THE RELATIONSHIP BETWEEN PLATE COUNTS OF MOLDS, YEASTS AND BACTERIA ON 70 SAMPLES FROM CREAMERIES EQUIPPED WITH INTERNAL BUTTER WORKERS.

Sample No.	Bacteria	Molds	Yeasts
1	100	0	0
2	200	0	2
3	500	0	0
4	500	0	9
5	600	5	0
6	1,000	0	0
7	1,000	0	0
8	1,000	0	0
9	1,000	0	0
10	1,200	0	1
11	1,500	0	4
12	1,500	0	0
13	1,700	0	0
14	1,700	0	0
15	2,000	1	1
16	2,000	0	0
17	2,600	12	0
18	3,000	3	2
19	3,000	1	3
20	4,000	1	11
21	4,000	0	0
22	4,000	0	0
23	4,000	1	10
24	4,000	0	3
25	4,000	0	0
26	5,000	1	0
27	5,000	0	0
28	5,000	0	0
29	5,000	0	0
30	5,000	0	0
31	6,000	1	2
32	6,000	0	0
33	7,000	0	0
34	7,000	0	0
35	8,000	0	32

Continued

Sample No.	Bacteria	Molds	Yeasts
36	8,000	0	0
37	8,000	0	1
38	8,000	0	0
39	8,000	0	0
40	8,000	40	50
41	9,000	6	230
42	9,000	0	2
43	10,000	5	240
44	11,000	0	79
45	13,000	1	0
46	13,000	0	85
47	13,000	0	0
48	14,000	0	4
49	14,000	0	0
50	15,000	30	20
51	17,000	0	5
52	18,000	0	2
53	18,000	0	0
54	19,000	0	0
55	21,000	0	0
56	23,000	4	21
57	23,000	0	11
58	24,000	0	2
59	26,000	0	12
60	30,000	0	59
61	36,000	0	0
62	42,000	50	1,370
63	43,000	17	104
64	135,000	2	20
65	160,000	20	740
66	335,000	0	56
67	360,000	0	4
68	364,000	2	213
69	640,000	5	28
70	over 6,000,000	50	570

ial examined from a joint in a worker of this type gave the following counts per gram:

Bacteria	Over	1,000,000,000
Yeasts		10,000,000
Mycoderms		1,300,000

This material does not show the same relationship between the numbers of the different kinds of microorganisms as does the manufactured butter, probably due to a different equilibrium existing between these organisms at the very edge of the material from which the contamination of the butter takes place.

Table 4 contains comparative counts on 70 samples from a number of creameries in which the churns are equipped with internal butter workers which are more easily kept in a sanitary condition. Results similar to those reported in Table 3 have been obtained on six other churns of the same type. If these results are representative the evidence indicates that the type of churn with internal butter workers produces a higher percentage of butter with low counts of microorganisms. The high numbers of microorganisms in the crevices of churns which are so difficult to sterilize due to the poor heat conductivity of the wood illustrate the unsanitary conditions which may develop in the wooden churns at present used by the creamery industry.

In the preceding tables a considerable amount of data

has been presented to show that there is little definite relationship between plate counts of yeasts and molds and bacteria in butter. It has been suggested that this lack of relationship may be due to a flora of bacteria which is resistant to pasteurization. A thorough study of the flora of high temperature pasteurization as used by the creamery industry in Canada is necessary before this point could be definitely settled. However, surveys made on Alberta creameries having difficulty with high counts have not shown high counts on the pasteurized cream. The evidence has been practically always suggestive of unsanitary equipment.

There are probably a number of factors which contribute to the condition bringing about a lack of correlation between the plate counts of bacteria and yeasts and molds in butter. The following factors may be involved:

(1) The comparative growth rates of microorganisms.

(2) The comparative resistance of microorganisms to the methods employed in the sterilization of creamery equipment.

Under favorable conditions the generation times of bacteria are much shorter than the generation times of yeasts and molds. It is reported that Escherichia coli will produce a new generation in approximately 20 minutes under favorable conditions. However, a generation rate as high as this with a mixed flora in butter does not seem probable. Yeasts re-

produce at a much slower rate and require at least three hours for a new generation under favorable growth conditions. Molds reproduce at a much slower rate still. Under favorable growth conditions it is easy to visualize the differences in growth which will take place in these groups of microorganisms. A high count of bacteria and a low count of yeasts and molds in butter may be due to exposing churning cream to temperatures favoring growth of bacteria subsequent to pasteurization which destroys all the yeasts and molds. The same condition might easily result when a butter with few or no yeasts and molds is exposed to high storage temperatures.

Many bacteria are much more resistant to pasteurization and equipment sterilization than yeasts and molds. Table 5 illustrates the relationship between the numbers of microorganisms in the same cream, raw and when pasteurized. In the raw cream the bacteria counts are very high compared to the yeast and mold counts. Pasteurization entirely destroyed the yeasts and molds as shown by the plate count but a number of bacteria survived.

The counts reported in Table 6 were made on water which was used to rinse the churn following sterilization. The sterilization procedure for the churn was as follows: hot water at 80° - 90° C. entered the churn and was revolved for

TABLE 5.

THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA OF RAW AND PASTEURIZED SAMPLES OF THE SAME CREAM.

Sample Number	PLATE COUNTS					
	Raw			Pasteurized		
	Molds	Yeasts	Bacteria	Molds	Yeasts	Bacteria
1	300	7,000	46,000,000	0	0	2,500
2	10	6,600	12,000,000	0	0	600
3	300	15,600	500,000,000	0	0	33,000
4	10	640	49,000,000	0	0	1,900
5	..	..	..	0	0	16,800
6	..	1,300	181,000,000	.	.	..

TABLE 6.

THE PLATE COUNTS OF YEASTS, MOLDS AND BACTERIA OF RINSE WATER FROM A STERILIZED* CHURN.

Sample No.	PLATE COUNTS		
	Molds	Yeasts	Bacteria
1	0	0	52,000
2	0	0	9,000
3	0	0	90,000
4	0	0	176,000
5	0	0	440,000

*Sterilization here does not imply the strict bacteriological meaning of the word.

twenty to thirty minutes. The hot water was then drained and the churn was rinsed with a measured quantity of cold water low in bacterial content, the plate counts of which are reported. It is evident that the contamination of butter made in this churn will vary depending upon the effectiveness of the churn sterilization. The yeast and mold count would be of little value in these cases as the yeasts and molds are entirely destroyed as shown by the plate count while a considerable number of bacteria still remain to contaminate the butter.

The application of the yeast and mold count to Canadian butter has resulted in lowering the counts of these microorganisms to such an extent that over 50 percent of the commercial butter in one province is reported to have yeast and mold plate counts of zero. It is urgent that an inquiry be made into the significance of a yeast and mold count of zero. It is realized that the application of the yeast count in the microbiological analysis of butter was more or less incidental to the mold count which was used to detect and eliminate moldy butter. It has been assumed since, however, that the yeast count combined with the mold count can be used as a measure of the microbiological quality of butter. This assumption is probably correct in some

cases but the results reported on preceding pages indicate that in many circumstances the yeast and mold count is not a reliable index of the bacteriological quality of butter.

This result is interesting in the light of the reports of investigators on the relationship between the yeast and mold count and the keeping quality of butter. Bouska and Brown (5) report no correlation between keeping quality and the yeast and mold counts. Macy and Richie (16) came to the conclusion that the yeast and mold count of individual samples does not serve as a reliable index of the keeping quality of butter.

These results suggest that the yeast and mold count is not reliable as a sole measure of the bacteriological quality of butter and should be supplemented by bacteria counts.

SUMMARY.

1. The data presented in this section indicate that the plate count of yeasts and molds is not always representative of the bacteriological quality of butter manufactured from cream pasteurized at 170°F. for 10 minutes.

2. There are at least two factors which may account for a condition of high counts of bacteria and low counts of yeasts and molds in Alberta creamery butter.

   (1) The yeasts and molds are entirely destroyed at the pasteurization temperatures employed. Some types of bacteria survive these temperatures and the attempts made to sterilize churns. As a result butter is manufactured which contains a number of bacteria and very few if any yeasts and molds. Temperatures favoring growth will result in high counts of bacteria and low or zero counts of yeasts and molds.

   (2) When conditions in pasteurized cream or butter are made favorable for growth, the bacteria (due to a shorter generation time) increase at a greater rate than yeasts and molds.

3. The inefficiency of the yeast and mold count as a sole measure of the bacteriological quality of butter becomes more significant when the combined plate count of yeasts and molds is reduced to zero.

PART THREE

THE GROWTH OF MOLDS IN AND ON BUTTER.

INTRODUCTION.

The growth of molds in and on butter has caused the creamery industry, in the past, a great deal of annoyance and economic loss. Many investigations are reported in the scientific literature on this topic. Macy (14) in a study of some of the factors influencing the growth of molds in butter reviews this literature in detail. It is apparent from a review of the literature that many of the investigators of the growth of molds in butter have not paid sufficient attention to the peculiar physical structure of butter (King 12). They have apparently left unquestioned some of the factors which regulate the growth of molds in butter. There are a number of factors which influence markedly the growth of molds both on the surface and in the interior of butter which are worthy of further investigation. The following factors will be considered here in some detail:

(1) Salt concentration.

(2) Oxygen content.

(3) Moisture conditions.

(4) Storage temperatures.

(5) Mold contamination.

The Effect of the Salt Concentration.

The inhibitory effect of salt on the growth of molds and other microorganisms is well recognized. It has been observed in the creamery industry, both experimentally and in practice, that salted butter does not become moldy as easily as unsalted butter. A number of statements have been made by investigators to the effect that butter containing from 2 to 3 percent of salt will not permit the growth of molds. The influence of salt on the growth of molds may be expected to vary considerably due to the variability of the salt tolerances reported for members of this group of microorganisms. Due to the fact that the salt contained in butter is dissolved in the water droplets, where the growth of microorganisms must necessarily take place, the effective salt concentration of butter depends upon the moisture content.

The exact nature of the distribution of moisture droplets in butter is still a matter of controversy. However, there is evidence that the droplets are more or less isolated which would hinder uniform distribution of the salt. In addition some salt crystals undoubtedly become coated with a layer of fat which makes it doubtful if the added salt is ever completely and uniformly dissolved when added

in the undissolved form. In a butter containing 15.5 percent of moisture, which is near the maximum amount allowed by law, the following are the theoretical percentages of salt in the contained water for various salt percentages in the butter:

Percent Salt in Butter	Percent Salt in the Contained Water
1	6.5
2	12.9
3	19.4

Thom and Shaw (26) report that species of Oidium, Alternaria and Cladosporium cannot develop in butter containing 2.5 percent salt. The same authors also report that only Penicillium chysogenum germinated when the salted agar used in this study contained 21 percent NaCl. These observations were made when the petri dishes were incubated in a water saturated atmosphere. In addition they noted that the growth of molds was better on salted agar than on butter containing the same concentration of salt.

It is apparent from this research that the salt necessary to inhibit the growth of molds in butter is much higher than can be incorporated in the commercial product. Commercial butter, however, may contain sufficient salt to act as a preventative if other conditions influencing mold growth are not optimum.

Salt has proved to be the most effective agent in the

prevention of mold growth on the parchment wrappers used on butter. Macy and Pulkrabek (15) have reviewed the literature on the prevention of mold on parchment paper. They report that soaking parchment in boiling saturated salt solution is the most effective means for the prevention of moldy wrappers.

Within the past few years a number of creameries have resorted to the use of dry parchment as received from the manufacturer without further treatment in the packaging of print butter. By the use of this method it is claimed that the treatment of liners can be eliminated and trouble from moldy wrappers reduced.

A number of experiments were made to test the effectiveness of dry wrappers in preventing the growth of molds on the wrappers. Samples of salted butter with low counts of molds were each divided into two portions, one of which was inoculated with mold spores from cultures picked from moldy parchment. Then both inoculated and uninoculated butters were wrapped in parchment paper treated by several methods and placed in a humid atmosphere at 15° C. which is a common storage temperature for butter in the retail trade. Tables 1 and 2 contain the results obtained on five samples of butter. All the samples reported in Table 2 and sample 1 in Table 1 were inoculated externally with mold spores after being wrap-

TABLE 1.

THE GROWTH OF MOLDS ON INOCULATED AND UNINOCULATED BUTTER WRAPPED IN PARCHMENT PAPER PREPARED IN FOUR WAYS. BUTTER STORED AT HIGH HUMIDITY AT 15°C.

Butter Number	Percentage of Moisture	Percentage of Salt	Molds per c.c.	Location of Molding	Sterile Wrapper		Hot Saturated Salt Solution		Cold Saturated Salt Solution		Dry Wrapper no treatment	
					3 wks	13 wks	3 wks	13 wks	3 wks	13 wks	3 wks	13 wks
1	14.7	2.5	2(lgr)	Wrapper	+	+	-	-	-	-	-	-
			"	Butter	-	+	-	-	-	+	-	-
	14.2	2.2	100,000	Wrapper	-	+	-	-	-	-	-	+
			"	Butter	-	-	-	-	-	-	-	-
2	13.7	1.7	0	Wrapper	+	+	-	-	-	-	-	+
			"	Butter	-	+	-	+	-	+	-	+
	13.2	1.6	100,000	Wrapper	-	+	-	-	-	-	-	+
			"	Butter	-	-	-	-	-	-	-	-
3	15.0	1.2	5 White	Wrapper	+	+	-	-	-	-	-	+
			"	Butter	-	+	-	+	-	+	-	+
	14.7	1.1	60,000	Wrapper	-	+	-	-	-	-	-	+
			"	Butter	-	-	-	-	-	-	-	-

TABLE 2.

THE GROWTH OF MOLDS ON INOCULATED AND UNINOCULATED BUTTER WRAPPED IN PARCHMENT PAPER PREPARED BY FOUR METHODS.
BUTTER STORED AT HIGH HUMIDITY AT 15° C. FOR EIGHT WEEKS.

Butter Number	Percentage of Moisture	Percentage of Salt	Plate Count of Molds per c.c.	Location of Molding	Control Sterile Wrapper	Hot Saturated Salt Solution	Cold Saturated Salt Solution	Dry Wrapper no treatment
1	13.5	1.2	10	Wrapper	-	-	-	-
			"	Butter	-	+	-	-
	13.6	1.2	380,000	Wrapper	+	-	-	-
			"	Butter	-	-	-	-
2	14.1	1.4	10	Wrapper	+	-	-	+
			"	Butter	+	-	-	+
	13.1	1.4	780,000	Wrapper	-	-	-	-
			"	Butter	+	-	-	-

ped in parchment.

The results show mold development on the sterilized wrappers on each butter, both inoculated and uninoculated, at the end of 13 weeks storage. No attempt was made to keep the parchment in a sterile condition when wrapping the butter. The results obtained on the sterilized wrappers serve as a check on conditions of growth in the experiment. No mold development was observed on parchment wrappers treated in hot and cold saturated brine. However, mold did develop on the surface of the butter in some instances. No mold growth was evident on dry wrappers at the end of 3 weeks but growth was present on the dry parchment in six samples at the end of 8 and 13 weeks.

The results obtained in these experiments indicate that dry wrappers can not be depended upon to protect butter from mold at the high humidity used. Parchment treated in hot and cold saturated brine did not support the growth of mold. However, butter wrapped in this manner did show growth on the surface of the butter in several instances. Mold growth on the surface of the butter was more frequent on the uninoculated butter in each experiment. This observation is significant in that there is an indication that the number of mold spores present in the butter appears to bear little re-

lationship to the subsequent development of moldy surfaces which confirms the work of Combes and Eckles (7).

The Influence of Aerobic Conditions.

It is well recognized that molds are aerobic as very few references have been made to the growth of molds under partial pressures of oxygen. Consequently an adequate supply of air is necessary for mold growth. The oxygen supply, therefore, helps to regulate the growth of molds in the interior and on the surface of butter.

The most common type of molding observed in the butter industry is observed on the surfaces of the butter or on the enclosing parchment paper and in the air spaces of poorly packed butter. In these positions mold growth is rapid and luxuriant, indicating an abundant supply of oxygen.

In addition to the surface types of moldiness, molds are occasionally seen to develop in the interior of the butter. This type of mold growth in butter can best be described as being discontinuous throughout the mass of the butter. The moldy areas present a smudgy appearance. A serious economic loss is always involved when butter becomes moldy in this manner. During the progress of this research three outbreaks of this type of mold growth were brought to

my attention, only one of which I was able to study in detail.

A detailed microscopic examination of the butter from moldy areas showed growth originating in what appeared to be either moisture or air droplets which were dispersed throughout the butter. Growth centred around these areas and frequently hyphae were seen which left the droplet and penetrated the butter for a considerable distance. In those cases in which the mycelia did not leave the droplet a very compact mass of mycelium was formed within the droplet. The growth of this mold in butter was not entirely vegetative, as spores were frequently seen. The mycelia of the mold were characterized by an olive green pigment. A mold, a species of Cladosporium, which has been reported by a number of investigators as occurring in butter, was isolated and identified as being the same mold which caused the moldy condition.

The growth of this mold in the interior of butter suggests the existence of aerobic conditions at localized areas in butter which permit mold growth. Investigations on the air content of butter by Pickerill and Guthrie (19) show that butter may contain from 0.5 to 14.9 percent of air by volume with an average of 4 percent. The results of Guthrie (9) show that well worked butter may contain a higher per-

centage of air than poorly worked butter. The exact distribution of the air in butter is not known but one may expect that droplets of water in butter will probably contain a certain amount of air and moisture will be present in air pockets which make it difficult to distinguish moisture droplets from air pockets when butter is examined with a microscope.

Samples of butter which had been heavily inoculated with spores of Penicillium isolated from moldy wrappers and stored at temperatures favoring growth were examined for evidences of growth in the interior. In no case was growth detected which may indicate that penicillium, a common cause of surface molding, will not initiate growth of macroscopic consequence in the interior of butter.

The factors which permit the growth of Cladosporium and prevent the growth of Penicillium in the interior of butter have not been determined but the rare occurrence of this type of mold growth presents two possibilities. Either the conditions favoring this type of mold growth are relatively rare or the mold species able to develop under the conditions present in the interior of butter occur rarely. The latter possibility has some evidence in its support as colonies of the Cladosporium type are seldom detected in Alberta butter (13). There is a factor, however, which may explain why Cladosporium colonies have not been found more often. The species of

Cladosporium which was isolated from moldy butter grew slowly on the malt medium which is used in the yeast and mold counts of butter. This was especially true when the medium was acidified to pH 3.5. When the moldy butter containing Cladosporium was plated, no growth of Cladosporium was evident in the usual five day incubation period.

The Effect of Moisture.

Favorable conditions of moisture must be present before mold growth of any consequence takes place on butter. A number of investigators have experienced difficulty in producing mold growth on salted butter. This difficulty is apparently due to conditions of low humidity. Combes and Eckles found that butter made from cream containing enormous numbers of mold spores and butter inoculated with a large number of spores failed to become moldy under the conditions which they employed. No mention was made regarding the humidity of the atmosphere or the salt content of the butter in these experiments. Thom and Shaw state that a relative humidity of less than 70 percent in the surrounding atmosphere will not favor the growth of molds. Tomkins (25) recently made a study of the growth of mold spores on surfaces at different humidities and temperatures. At 25° C. the spores of Alternaria citris germinated and the mycelium grew to a length of 7.7 microns

at 100 percent relative humidity and only 0.3 microns at 90.8 precent relative humidity. This research shows the striking effect of humidity on the growth of molds.

In my experiments on the growth of molds on butter the effect of humidity has been very noticable. Inoculated butters stored at 12° to 15° C. in an ice-box failed to develop mold in two months. Following their removal to a moist chamber at the same temperature, mold growth soon became apparent. In some instances inoculated and uninoculated butter placed immediately in moist chambers at the above temperature developed moldiness in from 2 to 3 weeks.

In the case of surface molding and mold growth on the parchment there seem to be two distinct conditions which will supply sufficient moisture for mold development. The first condition is the use of wet liners or wrappers which transfer large amounts of water to the surface of butter or any other practice in the packing or working process which results in a deposit of large amounts of water on the surfaces. In the second condition, the surface of the butter may not be moist immediately after manufacture but due to conditions of temperature and humidity during storage, a microscopic surface film of condensed moisture may form which will supply moisture for mold development. It is necessary to visualize the

distribution of moisture in butter in order to see more clearly the relationships involved in the growth of molds on its surface. Due to the peculiar distribution of moisture in butter (King (12), Rahn and Boysen (21)) the incorporated moisture of normal butter is probably not sufficiently available for the growth of molds on the surface. I have, therefore, postulated the presence of a condensation film as a possible mechanism favoring the growth of molds on the surface of butter which has not acquired such a film from some other cause. The presence of a parchment wrapper not in contact with the surface of packaged butter makes possible a film of moisture in the capillary space thus provided. A thin film would not be possible otherwise due to surface tension effects which cause moisture to form in droplets on the butter surface.

Due to the fact that moisture conditions are frequently made favorable for the growth of molds on the surfaces of butter, some methods must be used to prevent growth. The usual method employed to prevent mold growth on butter surfaces is the use of parchment wrappers which are boiled in a saturated solution of NaCl. A few of the results obtained on butters wrapped by this method are reported in Tables 1 and 2. The butters reported in these tables were stored in an atmosphere saturated with moisture at a temperature favorable for mold growth. Consequently surface mold growth was

not prevented when the butter was stored for periods of 8 to 13 weeks. When stored for a shorter period the results indicate that mold development was effectively prevented by dry wrappers and wrappers boiled in saturated salt solution.

The effectiveness of dry unsterilized parchment in preventing surface molding is probably due to two factors, viz.,

(1) No moisture is transferred to the surfaces as is the case with wet wrappers.

(2) Any moisture which forms tends to be absorbed, thus making conditions less favorable for growth.

The use of the dry unsterilized wrapper has proved effective in creamery practice but it is obvious that little protection is possible when the butter is stored at a very high relative humidity.

The Effect of Temperatures.

With few exceptions molds show optimum growth between 20° and 30° C., above and below which range growth is retarded. Macy reports that a number of species of mold which commonly cause butter to become moldy show slow growth at temperatures near the freezing point. In commercial butter storages, butter is stored at around 0° C. Tomkins reports that higher relative humidities are required for mold at temperatures on either side of the optimum temperatures. Sudden changes in the temperature of package butter should

be avoided as much as possible in order to prevent condensation of moisture which favors the formation of the condensation films already mentioned.

The Effect of the Extent of Contamination with Mold Spores.

Current opinion seems to imply a relationship between the number of mold spores contained in a sample of butter and the subsequent development of moldiness. Combes and Eckles, as already mentioned, show that butter containing a large contamination of mold spores failed to become moldy. The data in Tables 1 and 2 show that the butter containing few molds becomes moldy as quickly as highly contaminated butter. Other samples inoculated with mold spores failed to become moldy unless the butter was exposed to a high relative humidity. The results obtained indicate that a high humidity is more important for the subsequent development of molds on butter than the mold content of the butter.

Summary.

1. The literature on the growth of molds in butter has been reviewed and some experimental data have been presented on the factors influencing the growth of molds in and on butter.

2. The amount of salt necessary to inhibit the growth of molds in and on butter is too high to have any commercial application. However, some preventative effect may be possible through the use of salt.

3. The use of parchment paper boiled in a saturated solution of salt is effective in preventing the development of molds on parchment wrappers. Unsterilized dry parchment is effective for short periods of time but affords little protection under conditions of high humidity.

4. Two types of mold growth have been observed on butter. The common type is surface moldiness. A type which occurrs rarely, presents a discontinuous, smudgy appearance in the interior of butter. A mold, a species of Cladosporium, has been isolated as the causal organism in a churning of butter showing the interior type of mold growth.

5. A condensation film caused by changes in temperature has been postulated as a mechanism supplying favorable conditions of humidity for the growth of molds on butter surfaces in some instances.

6. The evidence obtained indicates that the conditions influencing the growth of molds are more important than the mold content on the subsequent development of molds in or on the surface of butter.

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